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Chemotherapeutic drugs delivered by systematic administration exhibit a great toxicity; patients have to endure the suffering from frequent injection. Thus, chronic and controlled release of chemotherapeutic drugs from biodegradable fibers implanted within the prostate tumor stroma will be a superior treatment modality. Furthermore, non-invasive and real time monitoring of dynamic response and chronic changes of the tumors to therapeutic interventions will help us better understand the therapeutic process, manipulate and optimize the therapeutic outcome.

The Hypotheses are (1) A near infrared (NIR) imager can non-invasively monitor the dynamic and chronic distribution of chemotherapeutic drug, vascular oxygenation, and blood volume in prostate tumors. (2) The dynamic response of prostate tumor oxygenation to the chronic drug delivery through the biodegradable fibers can serve as an indicator for treatment prognosis. The Specific Aims are 1) to design and implement an NIR spectroscopic imaging system suitable for dynamic imaging of drug concentrations, tumor oxygenation, and tumor blood volume, 2) to develop a 2D tomographic reconstruction algorithm, so as to obtain 2D tomographic images of tumor vascular oxygenation and the drug concentration delivered through the biodegradable fibers, 3) to load therapeutic drugs onto the biodegradable fibers and control the dosage and releasing rate, and 4) to study the relationship among the drug release rate, tumor oxygenation changes, and the therapeutic outcome, for obtaining optimal conditions in drug delivery.

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2004-2005 ANNUAL PROGRESS REPORT (YEAR 1)

This report presents the specific aims and accomplishments of our prostate cancer research project during the first year of funding sponsored by the U.S. Department of the Army. It covers our activities from March 1, 2004 to February 28, 2005.

Introduction

Radical prostatectomy is often favored for local disease, but it is associated with distinct risks of mortality and consequences, such as incontinence and sexual dysfunction. Non-surgical modality, such as chemotherapy, provides an alternative way for prostate treatment. However, the chemotherapeutic drugs delivered by systematic administration exhibit a great toxicity for the tissues that are with high growth fractions. And patients have to endure the suffering from frequent injection. Therefore, chronic and controlled release of chemotherapeutic drugs from biodegradable fibers implanted within the prostate tumor stroma will be a superior treatment modality compared to the conventional systematic injection. Non-invasive, real time monitoring of dynamic response and chronic changes of the tumors to therapeutic interventions will help us better understand the therapeutic process, further manipulate and optimize the therapeutic outcome.

The hypotheses for this proposed project are 1) a near infrared (NIR) imager can non-invasively, in real time monitor the dynamic and chronic distribution of chemotherapeutic drug, vascular oxygenation, and blood volume in prostate tumors simultaneously; 2) the dynamic response of prostate tumor oxygenation to the chronic drug delivery through the biodegradable fibers can serve as an indicator for treatment prognosis.

The project has four specific aims:
Aim 1: to design and implement an 8-source, 8-detector, near infrared (NIR) spectroscopic imaging system suitable for dynamic imaging of drug concentrations, tumor oxygenation, and tumor blood volume.
Aim 2: to develop a 2D tomographic reconstruction algorithm, so as to obtain 2D tomographic images of tumor vascular oxygenation and the drug concentration delivered through the biodegradable fibers.
Aim 3: to load therapeutic drugs onto the biodegradable fibers and control the dosage and releasing rate.
Aim 4: to study the relationship among the drug release rate, tumor oxygenation changes, and the therapeutic outcome, for obtaining optimal conditions in drug delivery.

Important Note on the Status of Principal Investigator

Although the funding was arrived at the University of Texas at Arlington in early Spring of 2004, the PI of the project was absent from January 2004 to May 31, 2004 due to the delayed return from P.R. China. This delay was caused by the required security check at the US Embassy in China when the PI came back from her Christmas vacation in China, and the official

notification was sent to the funding agency, i.e., the U.S. Department of the Army. Upon the return to the US in June 2004, the PI, Dr. Yueqing Gu, started immediately to work with her collaborators, Drs. Hanli Liu and Liping Tang, in two aspects: to develop the hardware for the NIR imaging system and to develop animal tumor models for the project.

While the research development was moving on schedule for 6 months, the original PI, Dr. Gu, determined to go back to her original institution in China in November, 2005. Therefore, for the last 3 months (Dec. 2004 to Feb. 2005), the two collaborators, Liu and Tang, have been continuing the development of both imaging hardware and animal models while the process in transferring the PI to a new qualified faculty member was taken place. Finally, the new principal investigator, Dr. Dan Popa, was identified and approved on Feb. 10, 2005 by Dr. Julie Wilberding from USAMRAA.

Therefore, this Year 1 report reflects the work that has been conducted in the last 8 months by the previous PI and her collaborators.

Body of the Report

1. Imaging system development

Task 1: To implement a Broadband NIR Imager (for Aim 1) (***months 1-14***)

a. To construct 8-source, 8-detector NIR Imager system;
b. To calibrate and test each channel of the new NIR Imager;
c. To optimize the distributions of sources/detectors and make a holder for the imager fibers.

Task 2: To employ the Finite Element Method and a non-linear least-squared optimization approach to obtain 2D cross-sectional maps of Hb, HbO and drug concentrations delivered based on the 8-channel NIR imaging measurements (for Aim 2) ***(months 1-14, in parallel with Task 1)***

a. To utilize the finite element method to conduct forward calculations for light propagation in the tumor tissues;
b. To develop an optimization algorithm using a non-linear, least-squared optimization method to reconstruct a 2D cross-sectional map of Hb, HbO and drug concentration based on the 8-channel NIR measurements.

In the past 8 months, the previous PI, Dr. Gu, and her collaborator Liu have made significant efforts for the proposed project:

1. have completed the design of the multi-source, multi-detector, NIR spectroscopic imaging system;
2. have designed and constructed the first-generation of laboratory phantoms for hemodynamic measurements;
3. have developed several reconstruction algorithms for 2D tomographic NIR imaging;
4. have utilized and tested the newly developed reconstruction algorithms for 2D NIR tomography using computer simulated data.

For tesk 1, we are modifying our existing multi-channel spectrometer by increasing the slit width for all 8 channels, adding L2 mirrors for each channel, and utilizing a stronger light source

(Ocean Optics, Dunedin, FL). We also determined to use the bifurcated fiber probes with 1-mm core fibers (InnovaQuaartz Inc., Phoenix, AZ) and a fast 1-to-16, optical switch box with the wavelength range of 600-900 nm. Figure 1 shows the multi-channel NIR imaging system.

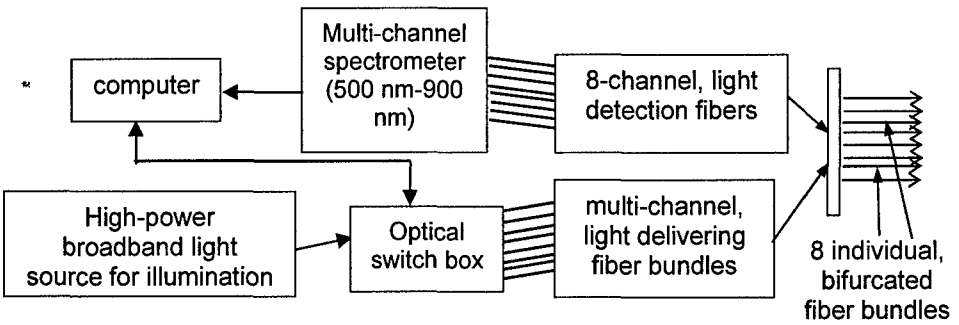


Figure 1(a). Schematic diagram of the multi-source, 8-detector transrectal NIR imaging system

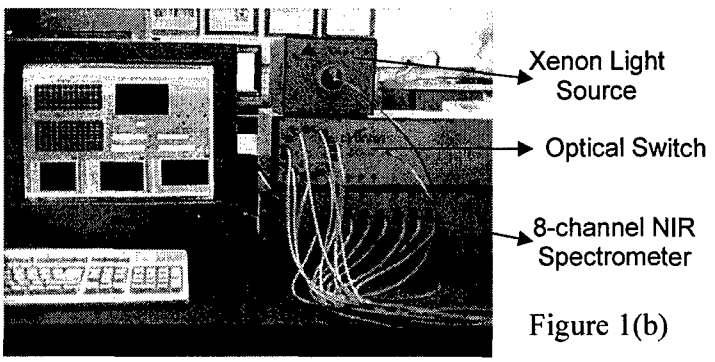


Figure 1(b)

As for a 8-source, 8-detector tomographic imaging system, we have used (1) 8 of the bifurcated fiber bundles, (2) an 1-to-8 optical switch box, (3) a broad-band CW Xenon light, and (4) an 8-channel spectrometer with CCD arrays with wavelengths of 500-900 nm. A complete NIR broadband imaging system is schematically drawn in Figure 1(a) above, and the actual setup is shown in Fig. 1(b).

We have completed the construction of first-generation, dynamic tissue phantoms, which allow us to have NIR absorption measured through simulated tissues while the flow rate of simulated blood passing through the phantoms. The two figures shown in Figs. 2(a) and 2(b) are the schematic diagram and the photo of the dynamic phantoms.

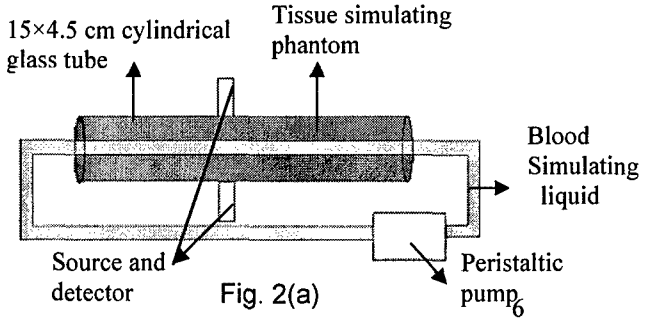


Fig. 2(a)

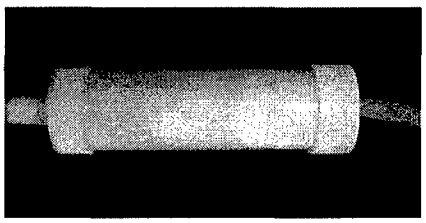
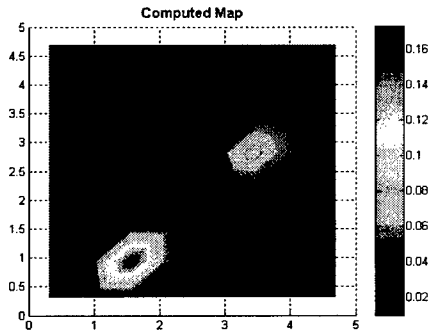


Fig. 2(b)

For Tesk 2, we have developed four reconstruction algorithms for 2D tomographic NIR imaging: a) nonlinear least-square algorithm (NLA), b) optimization and perturbation algorithm (OPA), c) nonlinear perturbation algorithm (NPA), and d) sequential quadratic programming with Gauss-Newton search algorithm (SQPGNA). Each of these algorithms has its advantages in converging/reconstructing the optical properties of measured sample.

We have tested the four newly developed reconstruction algorithms, using computer simulated data and performed noise analysis. Figure 3 shows an example of the reconstructed tomographic image, whereas Figure 4 displays a contour of error function and shows multiple local minimum points in the objective fuction.

Fig. 3



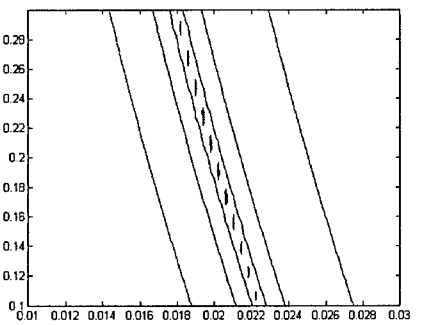


Fig. 4

2. Animal tumor model development

Besides working on Tasks 1 and 2, the previous PI has worked with both collaborators Liu and Tang, to develop right animal tumor models and choose right anti-cancer drugs. For this purpose, we have used 11 mice in a preliminary study, with human breast adenocarcinoma (Cell line HTB-26) implanted on 8 of them, and 3 were used as a control group. This cell line took around 3-6 months to develop into a size of 7-8 mm. The tumor was then implanted near back of the neck of the mouse to facilitate easy measurement setup. Nude mice were preferred as they have non-functioning T cells and thus unable to reject allografts or xenografts facilitating implantation of biodegradable fibers loaded with drugs.

Data acquisition of NIR readings was performed when the tumors were of 7-8 mm in length using ISS OxiplexTS™ oximeter. The OxiplexTS™ system allows the measurement of oxygenated and de-oxygenated hemoglobin concentrations in tumor tissue. The NIR system has eight sources with four emitting light at 750nm and the other four at 830nm. The emitted light diffused through the mouse tumor and was collected by a single detector. The setup for the study are shown in Figures 5(a). Isoflurane was used as the anesthetic agent, and the required amount was transferred to the anesthesia unit. A constant gas flow was supplied to the anesthesia unit, and the mouse was placed in the anesthesia chamber. When the mouse was anesthetized completely, they were transferred to the animal imaging setup. Here, anesthesia was provided through a nozzle and toggled between the anesthesia chamber and nozzle using a manifold.

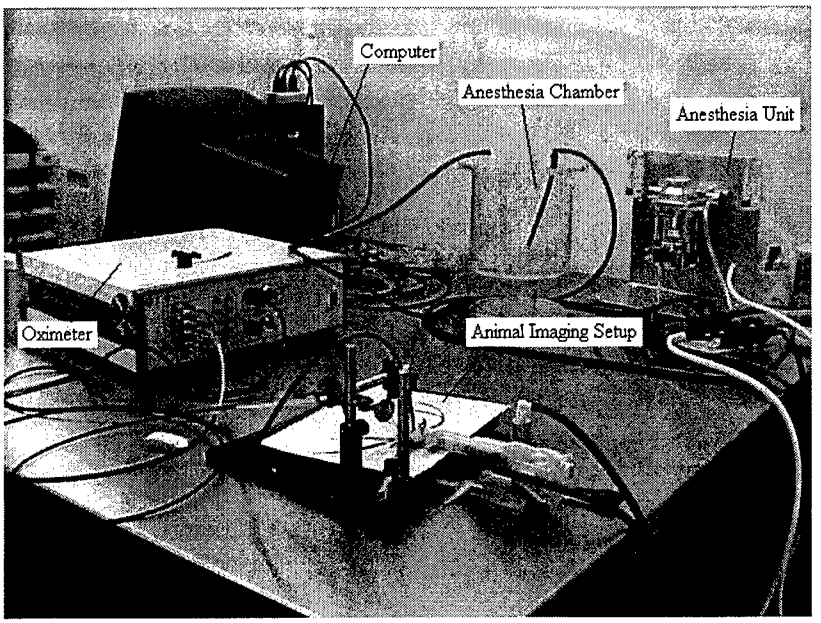


Figure 5(a). Setup for the tumor study

During NIR data acquisition, the source fibers were fixed in pairs of 750nm and 830nm at four locations using a circular probe holder designed for the study. The detector fiber was placed on the top and centered in such a way that equal amount of light is collected from each one of them. The animal imaging setup along with source-detector fibers and flow nozzle arrangement is shown in Figure 5(b).

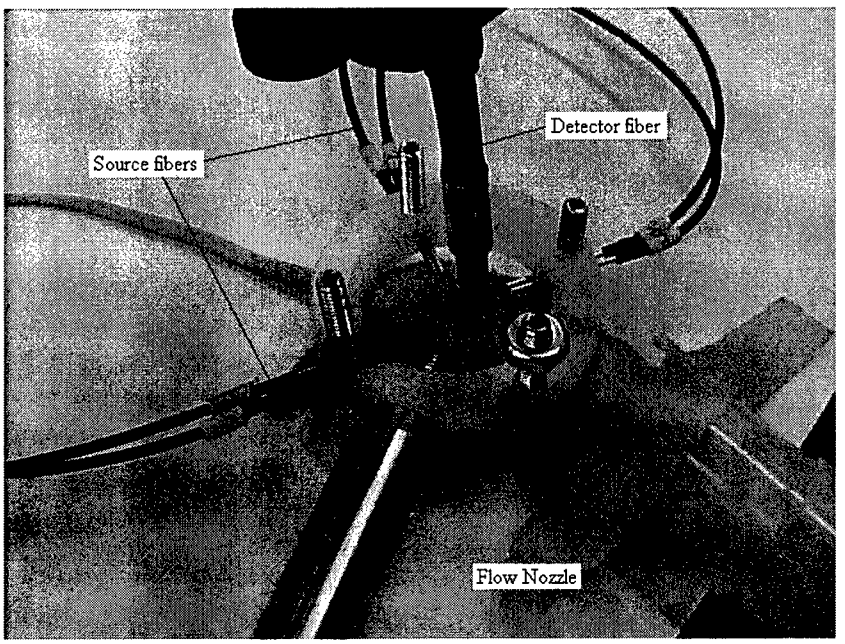


Figure 5(b). Animal imaging setup

The NIR measurements were made with air-oxygen intervention, first with air for 10 minutes, then followed by oxygen for 15 minutes, and again back to air for 15 minutes. The tumors were known to be hypoxic, so when the animal was provided with pure oxygen, the tumor vascular oxygenation tends to increase. The preliminary results obtained from one set of measurements at different locations are shown in Figures 6 through 9, where HbO_2 and Hb represent oxygenated and deoxygenated hemoglobin concentrations.

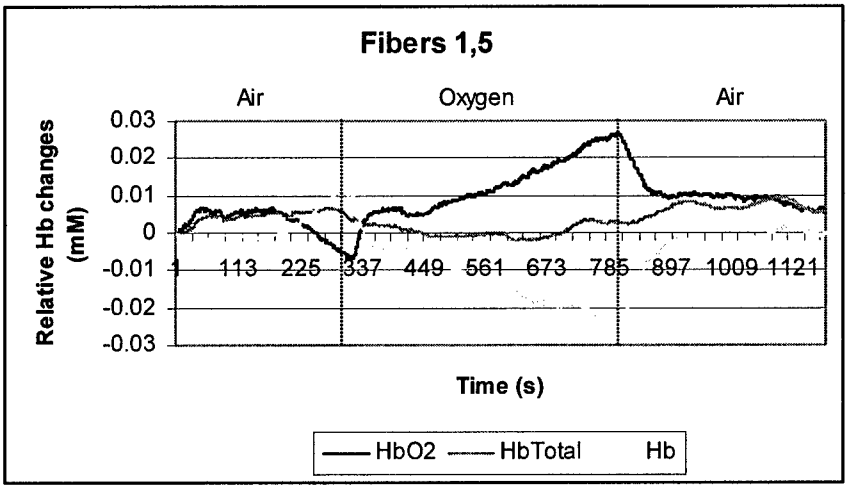


Figure 6. Data taken from fibers 1 and 5 at location 1

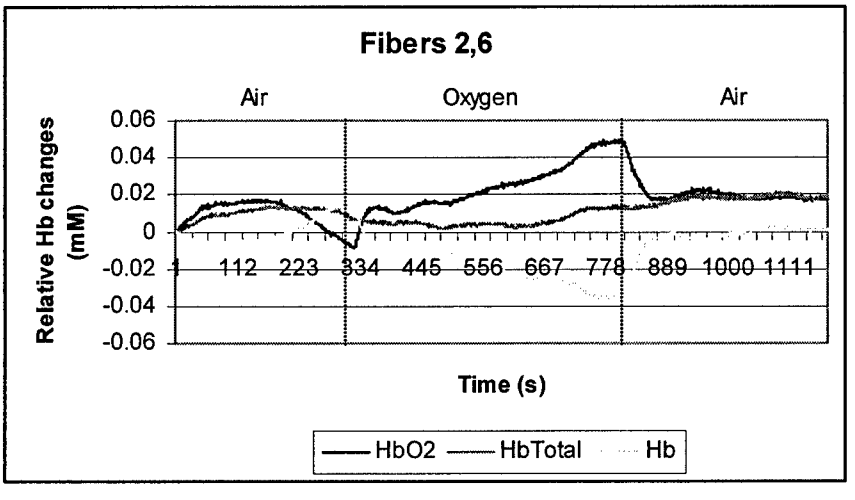


Figure 7. Data from fibers 2 and 6 at location 2

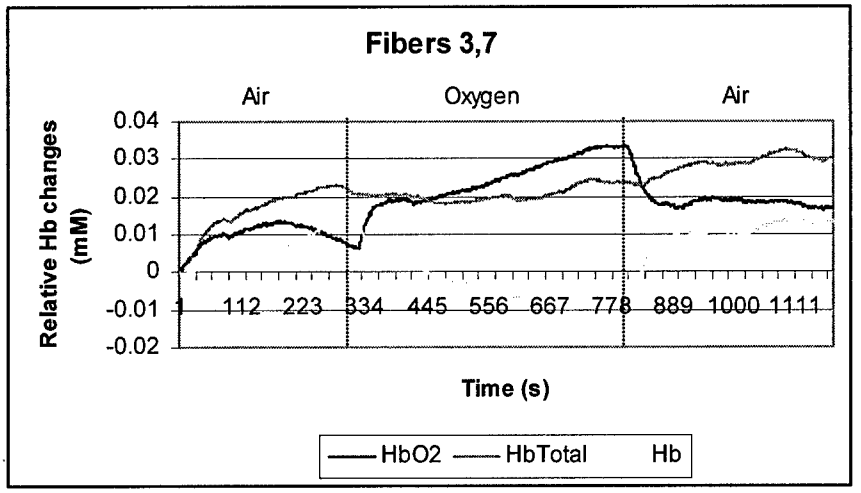


Figure 8. Data from fibers 3 and 7 at location 3

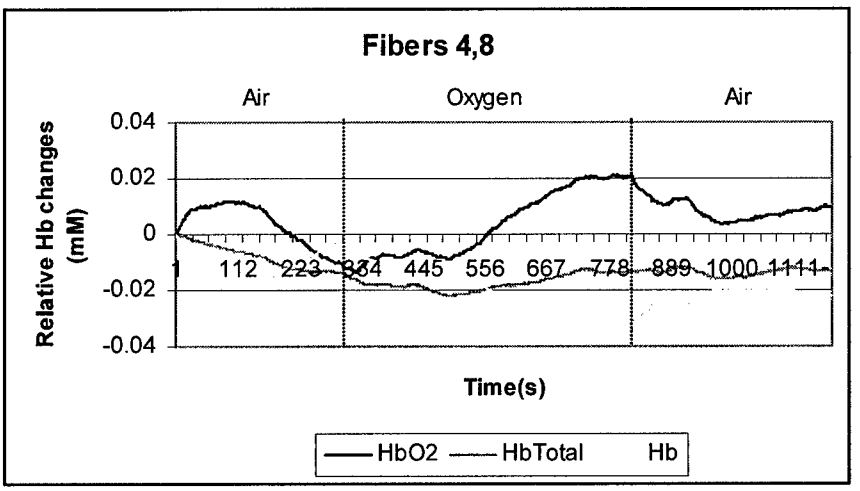


Figure 9. Data from fibers 4 and 8 at location 4

These figures demonstrate large changes in HbO_2 and Hb when the breathing gas for the animals was switched from air to oxygen. In another set of measurements, data did not seem to be as expected, or was with a lot of noise. This may be due to the variable distance between the different locations. When the distance was not equal, less amount of light reaches from that location, and hence no meaningful data can be retrieved.

Also, we have observed that the cell line used produced very hard tumors. For the next step, prostrate cell lines will be properly chosen and used. Furthermore, a couple of problems were rectified during this study. Firstly, there were problems with the gas intervention setup in the animal measurements. We have modified the previous setup that had lengthy tubes without values placed at required positions. Now, the flow nozzle in the animal imaging setup has improved for delivery of the gas to the mouse in full effect. Secondly, the room temperature was

very low for the mice under anesthesia, and further measurements will be made with mice kept under controlled temperature conditions using a warm blanket. In the immediately next step, drug 'Paclitaxol' will be injected in the mouse, and the data will be analyzed to examine changes due to drug injections.

Key Research Accomplishments

1) We have primarily implemented a broadband 8-source, 8-detector NIR imaging system that can be suitable for dynamic imaging of drug concentrations, tumor vascular oxygenation, and tumor blood volume for prostate tumors measured with local chemotherapy administration.
2) We have investigated a 2D tomographic reconstruction algorithm using computer simulations; such an algorithm can be used to obtain 2D tomographic images of prostate tumor vascular oxygenation and the drug concentration delivered through the biodegradable fibers.
3) Furthermore, we have conducted preliminary animal studies to test tumor types and tumor responses to oxygen interventions, to acquire preliminary data of NIR readings from the animals, and to examine drug dosages for corresponding tumors.
4) Finally, within the last 8 months, we have formed the collaborative team to work together for this proposed project while the PI has been in transition from one, Dr. Yueqing Gu, to another, Dr. Dan Popa. We believe that with the new PI in position, the collaborative effort will be continued with the original plans to be carried out actively in the coming 2 years.

Reportable Outcomes

Within the last 8 months, there is not yet any manuscript or proceeding paper developed based on the funded study. We expect to have such outcomes soon in the next reporting period.

Conclusions

The preliminary study that was carried out in the last 8 months has demonstrated that it is feasible to implement a broadband 8-source, 8-detector NIR imaging system that can be suitable for dynamic imaging of drug concentrations, tumor oxygenation, and tumor blood volume for prostate tumors under local delivery of chemotherapy. Our initial computer simulations have shown that a 2D tomographic reconstruction algorithm can be useful in obtaining 2D tomographic images of tumor vascular oxygenation and the drug concentration delivered through the biodegradable fibers. Furthermore, our preliminary animal studies provide us with confidence and evidence that animal tumors in response to oxygen interventions can be sensitively detected by an NIR imaging system, which can be essential to examine drug dosages and responses to therapy for prostate tumors.

List of personnel receiving pay from the research effort

Yueqing Gu,	Ph.D. the PI;	June 1, 2004 to Dec. 1, 2004
Woon Kyung Kim,	Graduate Research Assistant;	Sept. 1, 2004 to May 31, 2004
Vikrant Sharma,	Graduate Research Assistant;	Sept. 1, 2004 to May 31, 2004
Manan Goel,	Graduate Research Assistant.	January 14, 2005 to May 31, 2004